Bioreactors for surface-immobilized cells*

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Abstract

Surface immobilization of plant cells avoids the problem of hydrodynamic or shear stress, which tends to be characteristic of suspended cells cultured in typical, mechanically agitated bioreactor systems. Surface immobilization also promotes the natural tendency for plant cells to aggregate, which may improve the synthesis and accumulation of secondary metabolites. In addition, exchange of medium is made simple in surface-immobilized systems, and extracellular secondary products are easily recovered on a continuous basis. However, problems related to regulation of the thickness of the immobilized cell layer, maintenance of the biomass in a productive condition, and vacuolar retention of secondary products have yet to be resolved satisfactorily. This review focusses on two surface-immobilization technologies, differing primarily in the nature and the configuration of the inert support. Prototypes of these designs have been applied to a variety of plant cell systems at bioreactor volumes up to 20 litres. Results obtained with several alternative technologies are also summarized.

Abbreviations: 2,4-D – 2,4-dichlorophenoxyacetic acid, SIPCB – surface-immobilized plant cell bioreactor

Introduction

The term surface-immobilized plant cell bioreactor (SIPCB) applies only to those bioreactor systems where the biomass is retained (immobilized) on the exterior surface of an inert support without the aid of covalent attachment. The SIPCB is inoculated with a plant cell suspension of suitable density and operated for an initial period as a suspension bioreactor of air-lift or mechanically-agitated design, during which time virtually all of the cells spontaneously adhere or otherwise become attached to the surface of the inert support. Subsequent growth of the immobilized substratum is in a direction outward from the support in the form of a more or less continuous layer or tissue-like structure. This is in contrast to other plant cell immobilization technologies where the biomass is entrapped within the internal voids or pores of the supporting matrix or within a membranous or gelatinous structure.

The real and potential advantages of immobilized plant cell culture technology over free cell (suspension) culture have often been stated (Brodelius & Mosbach 1982; Lindsey et al. 1983; Payne et al. 1991; Yeoman 1987). Immobilized culture promotes the natural tendency for plant cells to aggregate or form tissues, thereby maximizing cell-to-cell contact. This may establish a more 'plant-like' physiology within the biomass, with a concomitant improvement in secondary metabolite synthesis and accumulation. In immobilized systems, the biomass is segregated from the culture medium. Consequently, the potentially damaging hydrodynamic (shear) stresses to which plant cells are subjected in suspension culture are avoided. This physical
separation of cells and medium also makes for easy exchange of medium for purposes of metabolic control or nutrient replenishment, and simplifies recovery of extracellular secondary products from spent medium. The composition of the culture medium can be readily and continuously monitored via an external loop, and the concentrations of \( \text{O}_2 \), sugar, etc. adjusted as required. Similarly, extracellular products can be harvested continuously by adsorption on a suitable resin, or by other means.

Surface immobilization affords a number of advantages over other plant cell immobilization technologies, which depend upon entrapment of biomass within a membrane or other porous structure such as foam cubes or gel beads. Perhaps of most significance is the absence of a SIPCB of any physical restriction to mass transfer between the culture medium and the biomass surface. The inherent simplicity of construction and operation of a SIPCB is also attractive. Since growth in a SIPCB occurs on the external surfaces of the support matrix, surface immobilization enables one to visually monitor the condition, distribution and extent of the biomass, and to routinely sample the biomass if desired.

In this review, attention will be focussed on two SIPCB designs differing primarily in the nature and configuration of the inert support, and referred to herein as Facchini-DiCosmo Surface Immobilization Technology (Facchini & DiCosmo 1991a, b) and Archambault-Volesky-Kurz Surface Immobilization Technology (Archambault et al. 1990a, b). Collectively, over the course of their development, prototypes of these two designs have been applied to a variety of plant cell systems at bioreactor volumes up to 20 litres (Archambault 1991). In the process, considerable insight has been gained into what is responsible for the adherence or attachment of plant cells to seemingly inert surfaces, and there now exists a substantial body of comparative data on the growth and productivity of suspended and surface immobilized cells.

**Technical requirements of a SIPCB system**

Many of the necessary attributes of a functional SIPCB system are common to other plant cell culture systems, free cell or immobilized. Accordingly, general design requirements of a SIPCB would include resistance to thermal and mechanical shock, thereby allowing sterilization with steam or dry heat. The bioreactor must also be able to maintain asepsis once achieved. Ports, connections, devices and instrumentation for continuous and/or periodic sampling, monitoring, medium exchange and aeration are required, as is some form of mechanical or air-lift agitation to aid initial attachment of the inoculum to the support and to minimize concentration gradients in the liquid phase throughout the culture period. The bioreactor should be equipped with a means of maintaining any desired operating temperature, and ideally would be transparent, at least in part, to allow visual monitoring of cell attachment and growth.

Requirements more directly related to cell immobilization would include a support matrix which is inexpensive, inert, and of the desired configuration, and which provides a suitably high surface to volume ratio without itself occupying a large proportion of the bioreactor volume. A large surface area permits high biomass loadings while minimizing the thickness of the biofilm/tissue layer on the support. Mass transfer restrictions within the biomass are thereby minimized since there is maximum contact between the surface of the immobilized culture and the liquid phase. A physiologically more uniform biomass would also be expected. The configuration and mixing characteristics of the bioreactor should promote rapid and complete immobilization of the inoculum, and the surface of the support matrix should be of such a composition and nature that the biomass remains immobilized in its entirety throughout the culture period.

There are a number of biological aspects to be considered if the benefits of surface immobilization technology are to be realized in the production of plant secondary metabolites. Perhaps most obvious is a requirement for the desired metabolite or metabolites to be released or excreted from the cell interior as a natural course of events, or that there exist some method of inducing product release without altering the viability or productivity of the biomass. Otherwise, product recovery will necessitate harvest of the biomass, negating the possibility of long term production of secondary metabolites by the immobilized culture with periodic or continuous harvest of the product from the liquid phase. Also required is a cultural protocol which limits growth of the cell layer to some optimum level, thus preventing an actively dividing culture from ‘overgrowing’ the support. Ideally, the biomass would then be held in an essentially non-dividing but metabolically active condition, wherein secondary metabolites are synthesized and subsequently released into the culture medium for some considerable period of time. Overgrowth is problematic with respect to mass transfer of nutrients into and metabolites out of the biomass, and...